FULL PAPER
THE ANALYST
www.rsc.org/analyst

Determination of diphenylamine stabilizer and its nitrated derivatives in smokeless gunpowder using a tandem MS method

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Received 20th December 2000, Accepted 16th January 2001
First published as an Advance Article on the web 2nd March 2001

A novel method for determination of diphenylamine (DPA) and its nitrated derivatives, which are considered as characteristic components in smokeless powder and gunshot residues, is described. A tandem mass spectrometric method is established and mass spectrometer parameters optimized for each compound to obtain higher sensitivity. Under optimum conditions, quantitative analysis was carried out. The linear ranges are 5.0–200.0, 2.0–200.0 and 5.0–250.0 ng ml^{-1} and the detection limits are 1.0, 0.5 and 2.5 ng ml^{-1} for diphenylamine (DPA), N-NO-diphenylamine (N-NO-DPA) and 4-NO_2-diphenylamine (4-NO_2-DPA), respectively. Intra-assay and inter-assay precision and accuracy of analysis of these three samples were investigated. Based on the regression lines obtained above, smokeless samples were analyzed. It was found that there are 0.952% DPA, 0.384% N-NO-DPA and 0.128% 4-NO_2-DPA in smokeless powder. Recovery tests showed that using cotton swabs, 80.3 ± 4.9% DPA, 79.6 ± 3.1% N-NO-DPA and 83.1 ± 5.4% 4-NO_2-DPA could be recovered from human hands.

1. Introduction

Smokeless gunpowders, used as modern propellant, are mainly composed of nitrocellulose and nitroglycerine. In many circumstances, particularly in moist air or under hot conditions, these two explosive ingredients can decompose to form nitric and nitrous acids, which will further degrade the gunpowder. Consequently, some stabilizers are used in smokeless gunpowders to deter such decomposition. Three commonly used stabilizers are diphenylamine (DPA), ethylcentralite (EC) and methylcentralite (MC). Identification of those stabilizers has become of great importance in forensic science because it can provide valuable evidence in firearm discharge cases.

Various methods have been developed to detect nitrocellulose[1–3] or nitroglycerine[4–7] in gunpowders and gunshot residues (GSRs). Unfortunately, these components have been regarded as being a lack of conclusive evidence. Nitrocellulose is widely used in varnishes, celluloid films, pharmaceutical industry, *etc.*[8] Nitroglycerine occurs in explosive as well as in pharmaceutical preparation. The analysis result of these compounds can be interfered with by sources other than gunpowders. In contrast, analysis for the stabilizers in smokeless powders can be very important to determine GSRs, and provides strong evidence for firearm discharge for forensic purposes.

EC and MC are regarded as characteristic materials in gunpowders.[9] Some papers have focused on the determination of EC and MC[3,10,11] and in our previous paper,[12] we have established a sensitive and selective MS-MS method to detect MC in smokeless powders and GSRs. However, many kinds of smokeless gunpowder use DPA as stabilizer instead of EC or MC. For these gunpowders, several papers[13–15] have considered the possibility that the detection of DPA may be taken as evidence. However, it should be of concern that DPA is used in rubber products and in the food industry, which will cause possible environmental contamination. Such contamination can be minimized if the nitrated derivatives of DPA can be determined. Those derivatives, arising from the reaction of DPA with the nitric and nitrous acids that result from the decomposition of nitrocellulose and nitroglycerin, are unique to smokeless gunpowders. Espinoza and Thornton[16] utilized TLC and LC to analyse DPA and its nitrated derivatives in gunpowders and achieved fairly good results. However, the sensitivity of TLC and LC techniques is inadequate considering that the amount of nitrated derivatives of DPA in some kinds of smokeless powders can be very low. It is necessary to establish a new method to obtain a higher sensitivity.

This paper focuses on the analysis of DPA and its four derivatives, including N-NO-DPA, 4-NO_2-DPA, 4-NO-DPA and 2,4-2NO_2-DPA. Fig. 1 shows the structures of these compounds. A tandem mass spectrometric method was established to identify the existence of these compounds in smokeless powder. The mass spectrometry conditions were

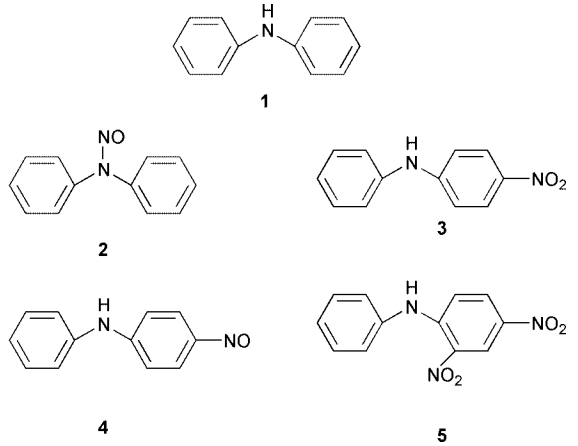

Fig. 1 Structures of DPA and its four nitrated derivatives, N-NO-DPA, 4-NO_2-DPA, 4-NO-DPA and 2,4-2NO_2-DPA. (**1**) Structure of DPA; (**2**) structure of N-NO-DPA; (**3**) structure of 4-NO_2-DPA; (**4**) structure of 4-NO-DPA; (**5**) structure of 2,4-2NO_2-DPA.

 DOI: 10.1039/b010183o

optimized. To improve the sensitivity and eliminate possible interference, a multiple reaction monitoring (MRM) mode was utilized. In the experiment, we detected two derivatives (N-NO-DPA and 4-NO_2-DPA) together with DPA. The sensitivity, intra-assay and inter-assay of the method were tested. In addition, the recoveries of these materials from cotton swabs were examined. It can be shown from the results that the proposed method is both sensitive and selective.

2. Experimental

2.1 Apparatus

The PE Sciex API 3000 triple quadrupole mass spectrometer (Thornhill, Ontario, Canada) fitted with an electrospray ionization (ESI) interface was utilized. Ultra-purity nitrogen gas was used as nebulizer, curtain and collision gas. All the parameters as mentioned in Table 1 were optimized in the experiment to improve the sensitivity of detection.

A flow-injection system was used throughout in our experiment to deliver samples. The flow rate was 0.1 ml min^{-1}. A column (C_{18}, 2.1 $\times$ 50 mm), maintained at room temperature, was installed before the sample injector to keep the flow rate stable and therefore keep the ion current stable.

2.2 Materials and reagents preparation

DPA, 4-NO-DPA, 4-NO_2-DPA and 2,4-2NO_2-DPA were purchased from Acros (New Jersey, USA). N-NO-DPA was purchased from Fluka (Switzerland). Smokeless gunpower was obtained from the lab of the Arm Police Institute (Langfang, China). Methanol was purchased from Fisher Scientific (Fair Lawn, New Jersey, USA).

All the solutions in the experiment were prepared with methanol. Stock standard solutions of DPA, N-NO-DPA, 4-NO_2-DPA, 4-NO-DPA and 2,4-2NO_2-DPA were prepared by dissolving 0.015 g of each compound in 50 ml methanol, respectively, and then diluting with methanol to give concentrations of 3 μg ml^{-1}. Calibration standards were freshly prepared daily for each assay by serial dilutions of stock standards. The stock powder solution was prepared by dissolving 0.100 g smokeless powder in 100 ml methanol to give a concentration of 1 mg ml^{-1}. Smokeless powder solutions used in the experiment were diluted from this stock solution to give concentrations of 10 μg ml^{-1}.

2.3 Procedures

After the operating conditions of the mass spectrometer were optimized, the mode of MRM for tandem MS was applied. Under this mode, 20 μl of gunpowder solution was injected into the mass spectrometer through a six-way valve to test the existence of DPA and its nitrated derivatives. Tests of calibration curve and recovery from cotton swabs were carried out under the same mode.

3. Results and discussion

3.1 Optimization of tandem MS conditions

MRM mode was utilized for determination of DPA, N-NO-DPA, 4-NO_2-DPA, 4-NO-DPA and 2,4-2NO_2-DPA in smokeless powder. Under this mode, a molecular ion of a compound was selected at the first quadrupole, and then after collision-activated dissociation, one of its characteristic product ions was selected by a second quadrupole. Ions that are not related to the target compound are filtered out through such a process. Thus possible interferences were eliminated. To use this mode, a pair of precursor/product ions should be chosen for each compound. Fig. 2 shows the full-scan mass spectra (positive ion mode) of these five compounds. Both molecular ions and product ions can be observed from the spectra. Take the spectra of DPA for example, *m/z* 170 is the molecular ion $[M+H]^+$ and *m/z* 93 is the most abundant product ion $[M-C_6H_5+H]^+$. Therefore, the precursor/product ion pairs were selected according to Fig. 2. In our experiment, *m/z* 170/93, 199/169, 215/198, 199/181 and 260/243 are selected for DPA, N-NO-DPA, 4-NO_2-DPA, 4-NO-DPA and 2,4-2NO_2-DPA, respectively.

Optimization of the mass spectrometer parameters should be carried out in order to improve the sensitivity for every specific compound. There are two procedures that enable this to be achieved. First, parameters, including nebulizer gas (NEB), curtain gas (CUR), ion spray voltage (IS), orifice voltage (OR), ring focus voltage (RNG), voltage of the first of quadrupole rod (RO_1), were adjusted to improve the intensity of precursor ion. Second, the flow rate of collision gas (CAD), voltage of collision range (RO_2), voltage of the second quadruple rod (RO_3) and those voltages of focusing lens were adjusted. The purpose of this procedure is to achieve a higher performance of collision. Through these procedures, more intensive selected product ions were obtained, which greatly improved the

Table 1 Optimized conditions of the mass spectrometer

Mass spectrometer conditions: (PE Sciex API 3000)					
Ionization mode	ESI, positive ion mode				
Scanning mode	Monitoring multiple reaction (MRM)				
Precursor/product ion pair	*m/z* 170/93 for DPA				
	m/z 199/169 for N-NO-DPA				
	m/z 215/198 for 4-NO_2-DPA				
	m/z 199/181 for 4-NO-DPA				
	m/z 260/243 for 2,4-2NO_2-DPA				
Parameters[a]	DPA	N-NO-DPA	4-NO_2-DPA	4-NO-DPA	2,4-2NO_2-DPA
NEB	2	4	3	3	4
CUR	12	12	12	12	12
CAD	4	4	6	6	4
IS	4500 V	4800 V	4500 V	4500 V	4800 V
OR	56 V	31 V	46 V	51 V	46 V
RNG	220 V	130 V	200 V	200 V	180 V
RO_1	−10.5 V	−11 V	−11 V	−11 V	−11 V
RO_2	−46 V	−26 V	−32 V	−44 V	−32 V
RO_3	−48 V	−28 V	−34 V	−46 V	−34 V

[a] NEB = nebulizer gas; CUR = curtain gas; CAD = collision gas; IS = ion spray voltage; OR = orifice voltage; RNG = ring focus voltage; RO_1 = voltage of 1st quadrupole rod; RO_2 = voltage of collision range; RO_3 = voltage of 2nd quadrupole rod.

sensitivity of the method. Table 1 lists the optimized conditions for each compound. Under these conditions, the following experiments were carried out.

3.2 Analysis of smokeless powder

This procedure is designed to investigate the existence of DPA and its four nitrated derivatives in selected smokeless gunpowder. Under the conditions listed in Table 1, 20 μl gunpowder sample of 10 μg ml^{-1} was injected into the mass spectrometer. The flow injection system was composed of a pump, a six-way valve with a 20 μl loop and a C_{18} column. The flow rate was 0.1ml min^{-1}.

Fig. 3 shows the analysis result. There was no interference observed in the blank samples with this MRM mode. DPA and its two nitrated derivatives, N-NO-DPA and 4-NO_2-DPA, were detected in the gunpowder sample. The signal-to-noise ratios (S/N) of these compounds were 89.7, 112.9 and 52.2, respectively. The other two nitrated derivatives, DPA, 4-NO-DPA and 2,4-2NO_2-DPA, were not found in the gunpowder sample. This may because 4-NO-DPA is a relatively reactive and unstable compound, and 2,4-2NO_2-DPA is hard to form during the reaction of DPA when there is little nitric and nitrous

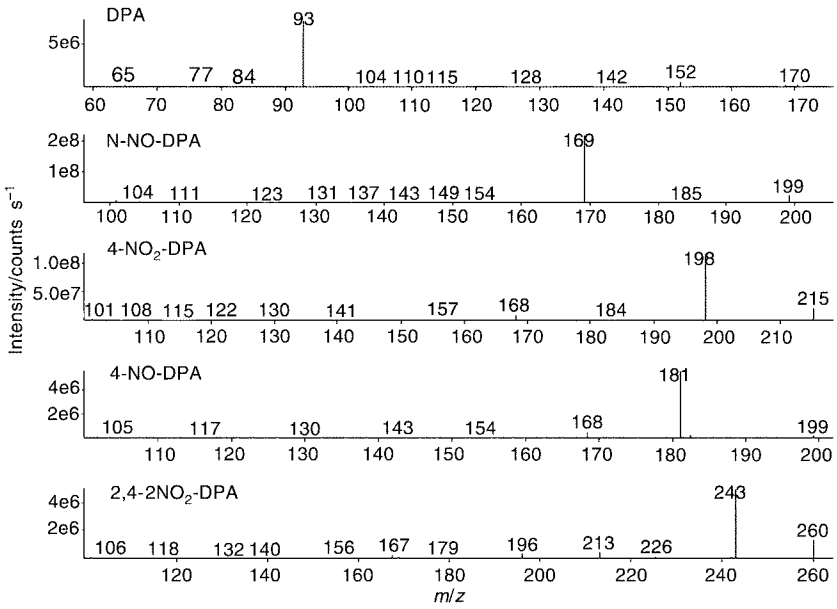

Fig. 2 The full-scan positive-ion mass spectra of DPA and its four nitrated derivatives after optimisation of the mass spectrometer parameters.

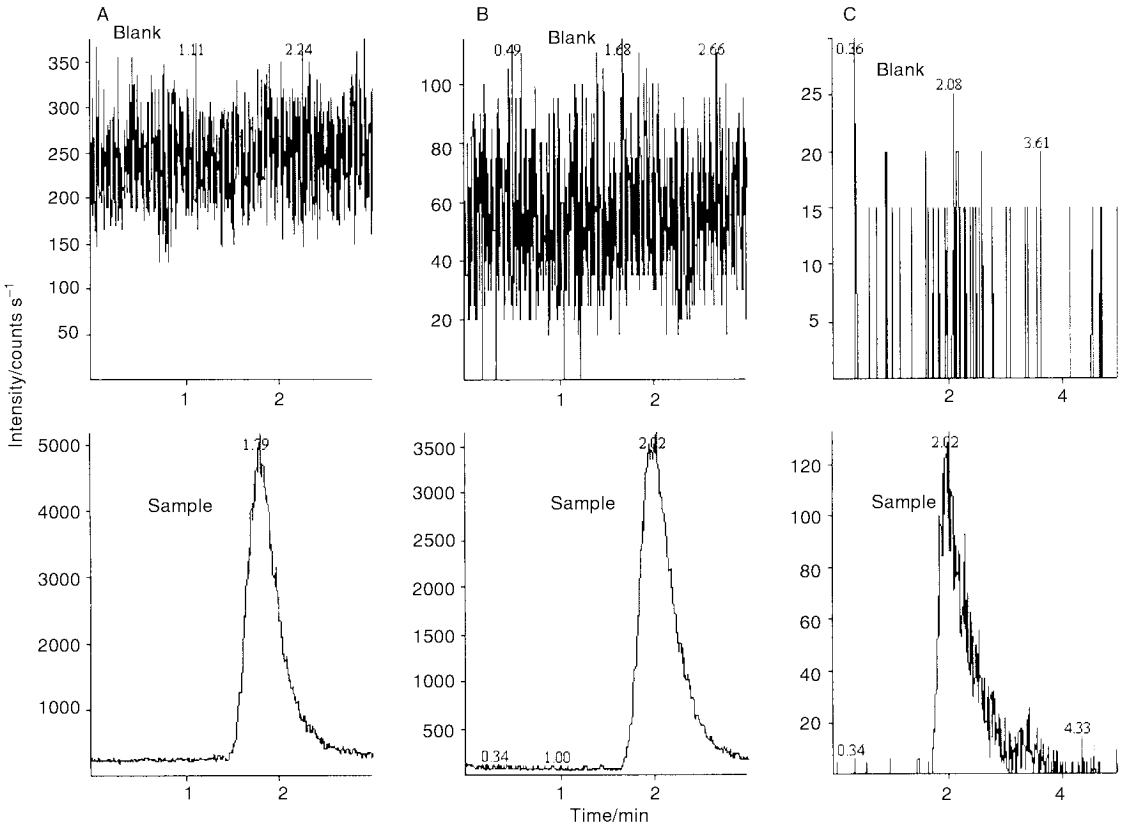

Fig. 3 Spectra of blanks and smokeless powder samples. A, Spectra of blank and DPA contained in powder sample. B, Spectra of blank and N-NO-DPA contained in powder sample. C, Spectra of blank and 4-NO_2-DPA contained in powder sample.

acids produced in the decomposition of nitrocellulose and nitroglycerin, especially when this decomposition is not so intensive. This conclusion corresponds with Espinoza and Thornton's results[16] that N-NO-DPA and 4-NO_2-DPA, in contrast to 4-NO-DPA and 2,4-2NO_2-DPA, are the two most commonly nitrated DPA occurring in gunpowders.

3.3 Quantitative analysis

The quantification of DPA and its derivatives in gunpowders was carried out in the present study. Under the optimal conditions described above, serial calibration standard solutions of DPA were analyzed. The results showed that the calibration graph of peak area (A) against the concentration of standard (C, ng ml^{-1}) was linear in the range of 5.0–200.0 ng ml^{-1} (A = 590.6C + 617.4, r^2 = 0.9997, n = 5), and the detection limit concentration is 1.0 ng ml^{-1}.

For N-NO-DPA, the calibration graph of peak area (A) against the concentration of standard (C, ng ml^{-1}) was linear in the range of 2.0–100.0 ng ml^{-1} (A = 2098C − 892.5, r^2 = 0.9992, n = 5), and the detection limit concentration is 0.5 ng ml^{-1}.

For 4-NO_2-DPA, the calibration graph of peak area (A) against the concentration of standard (C, ng ml^{-1}) was linear in the range of 5.0–250.0 ng ml^{-1} (A = 39.59C − 82.22, r^2 = 1.000, n = 5), and the detection limit concentration is 2.5ng ml^{-1}.

The data of intra-assay and inter-assay precision and accuracy for the analysis of the three compounds were obtained from analysis of standard samples in different concentrations within 4 days. Results are listed in Tables 2 to 4. The precision data was assessed by the coefficient of variation from analysis of measured samples and the accuracy data was expressed with the mean concentration and standard deviation from analysis of samples.

Following the same procedure for determining standard solutions, 10 μg ml^{-1} gunpowder samples were analyzed. Based on the calibration graph obtained above, the amount of DPA, N-NO-DPA and 4-NO_2-DPA in gunpowder samples was determined. The results are listed in Table 5. Peak areas and calibration graphs gave the concentrations of these three compounds in gunpowder solutions. Mean concentration values were divided by the concentration of gunpowder solution, 10 μg ml^{-1}, to give the content of gunpowder.

3.4 Recovery tests from hand

To test the recoveries of DPA from hand, 0.1 ml of 1 μg ml^{-1} DPA standard sample was spread on the human hand. After it was evaporated to dryness, a cotton swab soaked with methanol was used to extract DPA from the hand. A medical syringe was used to squeeze the cotton swab and then dilute it to 1.0 ml, 20 μl of which was injected into the mass spectrometer. The sample was quantified based on the regression equation obtained in the previous test. The recoveries of N-NO-DPA and 4-NO_2-DPA were tested using similar procedures. The analysis results of these three compounds are listed in Table 6. In addition, pure methanol was spread on the human hand and extracted in the same way to obtain blank samples. No obvious interferences were observed.

4. Conclusion

This paper describes a highly sensitive and selective method for the determination of trace amounts of DPA and its nitrated derivatives in smokeless gunpowder. In smokeless gunpowder tested in this experiment, DPA and its two mononitrated derivatives, N-NO-DPA and 4-NO_2-DPA were detected. The method is promising for the detection of other multinitro-DPA in certain gunpowders having been stored under hot or wet conditions, in which the decomposition of nitrocellulose and nitroglycerin is much more intensive. Furthermore, this paper provides a new method that could be applied to the determina-

Table 2 Intra-assay and inter-assay precision and accuracy of analysis of DPA samples

Concentration/ng ml^{-1}	Intra-assay precision (RSD) (%)	Intra-assay accuracy (mean±s, ng ml^{-1}) (n = 5)	Inter-assay precision (RSD) (%)	Inter-assay accuracy (mean±s, ng ml^{-1}) (n = 4)
10	11.2	10.7 ± 1.2	13.7	9.5 ± 1.3
20	7.8	20.6 ± 1.6	9.7	20.7 ± 2.0
50	5.7	51.0 ± 2.9	3.4	52.5 ± 1.8
100	1.4	97.7 ± 1.4	3.6	98.2 ± 3.5
200	1.1	200.8 ± 2.2	1.8	200.4 ± 3.6

Table 3 Intra-assay and inter-assay precision and accuracy of analysis of N-NO-DPA samples

Concentration/ng ml^{-1}	Intra-assay precision (RSD) (%)	Intra-assay accuracy (mean±s, ng ml^{-1}) (n = 5)	Inter-assay precision (RSD) (%)	Inter-assay accuracy (mean±s, ng ml^{-1}) (n = 4)
10	8.7	10.4 ± 0.9	10.5	10.5 ± 1.1
20	6.3	20.5 ± 1.3	7.0	19.9 ± 1.4
50	3.5	47.9 ± 1.7	2.5	51.6 ± 1.3
100	2.1	100.8 ± 2.1	1.5	99.2 ± 1.5

Table 4 Intra-assay and inter-assay precision and accuracy of analysis of 4-NO_2-DPA samples

Concentration/ng ml^{-1}	Intra-assay precision (RSD) (%)	Intra-assay accuracy (mean±s, ng ml^{-1}) (n = 5)	Inter-assay precision (RSD) (%)	Inter-assay accuracy (mean±s, ng ml^{-1}) (n = 4)
10	8.3	10.9 ± 0.9	8.0	11.3 ± 0.9
25	6.5	24.5 ± 1.6	6.0	24.9 ± 1.5
50	3.4	50.5 ± 1.7	3.8	52.1 ± 2.0
100	2.3	99.6 ± 2.3	2.4	101.2 ± 2.4
250	1.8	250.1 ± 4.5	1.5	251.7 ± 3.8

tion of DPA and nitrated DPA in gunshot residues. If both DPA and some of its nitrated derivatives can be detected in gunshot residues simultaneously and the relative content determined, this should provide important information to forensic scientists.

Table 5 Quantitative analysis of smokeless gunpowder samples

Compound	Concentration in gunpowder solutions (mean±*s*, ng ml^{-1}) (n = 5)	Content in smokeless gunpowder (%)
DPA	95.2 ± 2.1	0.952
N-NO-DPA	38.4 ± 1.8	0.384
4-NO_2-DPA	12.8 ± 4.3	0.128

Table 6 Results of recovery test from human hands

Compound	Recovery (mean±*s*, %) (n = 5)
DPA	80.3 ± 4.9
N-NO-DPA	79.6 ± 3.1
4-NO_2-DPA	83.1 ± 5.4
Blank	n/s

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